kallikrein preparation was inhibited by these agents. The chemotactic response was consistent with the generation of a C5a-like peptide from C5, because the effect could be partially inhibited by carboxypeptidase N and was related to the generation of a small (~14,000 mol wt) fragment of C5. In contrast, no chemotactic activity could be demonstrated when the zymogen prekallikrein was tested with C5 under identical conditions. Chemotactic activity was also generated when rabbit C5 was incubated with the 80,000-M, form of activated Hageman factor, trypsin or EAC423. No chemotactic activity was produced when C5 was absent from the incubation mixtures or when intact C5 alone was assayed. In sum, these results suggest the existence of a novel interaction between the Hageman factor and complement systems which may have biological relevance.

Wiggins, R. C., Giclas, P. C. and Henson, P. M. *(Cochrane, C. G.)*

Journal of Experimental Medicine 133:1391-1404, 1981.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunopathology, Research Institute of Scripps Clinic, La Jolla, CA.; and the Department of Pediatrics, National Jewish Hospital, and Departments of Medicine and Pathology, University of Colorado Medical School, Denver.

GUINEA PIG HAGEMAN FACTOR AS A VASCULAR PERMEABILITY ENHANCEMENT FACTOR

In this ongoing attempt to ascertain the biological role of the contact (Hageman factor) system, Hageman factor was purified from guinea pig plasma by successive column chromatography, and an active Hageman factor, β-HFa, was prepared for study. The guinea pig Hageman factor appeared homogeneous as a single-chain protein on polyacrylamide gels in the presence of sodium dodecyl sulfate and β-mercaptoethanol. Amino acid composition of the guinea pig Hageman factor was similar to that reported for human, bovine, and rabbit Hageman factors. The purified guinea pig Hageman factor, as well as guinea pig plasma, showed strong clotting time correction activity in Hageman-factor-deficient human plasma. The activity could be blocked by the IgG fraction of antiserums against guinea pig Hageman factor raised in rabbits or a goat. The concentration of Hageman factor in guinea pig plasma was determined to be 120 μg/ml by quantitative radial immunodiffusion assay. When β-HFa, the 28,000-dalton active form of Hageman factor, was prepared from guinea pig Hageman factor by treatment with plasma kallikrein, β-HFa caused an increase in vascular permeability when injected into guinea pig skin at concentrations as low as 3×10^{-10} M. This increased permeability was short-lasting, and the permeability enhancement activity of β-HFa was inhibited by pretreatment of β-HFa with diisoprophylfluorophosphate. According to the authors, it may be concluded, therefore, that active Hageman factor in the interstitial space of guinea pigs acts as a vascular permeability factor of far greater potency than bradykinin.

Yamamoto, T. and *Cochrane, C. G.*

American Journal of Pathology 105(2):164-173, 1981.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunopathology, Research Institute of Scripps Clinic, La Jolla, CA.

MODULATION OF POKEWEED-MITOGEN-INDUCED IMMUNOGLOBULIN SECRETION BY HUMAN BRONCHOALVEOLAR CELLS

The effects of pulmonary alveolar macrophages (PAM) on immunoglobulin (Ig) secretion were investigated, using autologous peripheral blood lymphocytes as the indicator population and pokeweed mitogen (PWM) as a monocyte-dependent polyclonal B-cell activator. Bronchoalveolar cells (BAC) from seven nonsmoking subjects suppressed the response to PWM by unfractionated autologous peripheral blood mononuclear cells (MNL), whereas low concentrations of BAC partially reconstituted the response of monocyte-depleted MNL to PWM. Thus, it could be seen that BAC could modulate PWM-induced Ig secretion in different ways, depending on the presence or absence of monocytes in the mononuclear cell population. The suppressor activities of BAC were not abrogated by prior irradiation and were only partially reversed by the addition of indomethacin to the cultures. However, prior disruption of BAC completely abolished their suppressive functions. Suppression of PWM-induced Ig secretion is probably mediated by intact, radioresistant PAM.

Lawrence, E. C., Theodore, B. J. and Martin, R. R.

American Review of Respiratory Disease 126:248-252, 1982.

Other support: American Lung Association and the National Institutes of Health.

From the Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Research Center of the Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

DEFECTIVE IMMUNOGLOBULIN SECRETION IN RESPONSE TO POKEWEED MITOGEN IN SARCOIDOSIS

Several previous studies of sarcoidosis have indicated a dichtomy between enhanced humoral immune functions clinically and defective *in vitro* B cell responsiveness, which suggested some disorder of immunoregulation. In the study presented here, it was found that polyclonal immunoglobulin (Ig) secretory response to pokeweed mitogen (PWM) — a plant lectin which requires the presence of both monocytes and T cells in order to trigger B cells — was defective as well. Specifically, *in vitro* immunoregulation of Ig secretion was studied in 21 patients with sarcoidosis. While peripheral blood mononuclear cells from normal individuals responded to PWM with a 10-fold or greater increment in Ig-secreting cells, cells from sarcoid patients failed to respond to PWM at any concentration employed. More monocytes were found in sarcoid mononuclear cell preparations (44.8±2.0% vs 30.4±1.4% in normal donors), but removal of monocytes improved the response to PWM in only four patients. Mononuclear cells from seven of 19 patients suppressed Ig secretion in co-cultures with normal donor cells. Patients exhibiting excessive suppressor cell function were older, with longer standing and less clinically active disease than non-suppressing patients. Monocyte removal reversed the suppression in only four of the suppressor patients, but excessive suppressor monocyte function was later demonstrated in two sarcoid patients whose cells initially did not suppress Ig secretion when cultured with normal cells. While the immunological defects in sarcoidosis may be complex, heterogenous and

dynamic, these data suggest that suppressor monocytes, when present in sarcoidosis, may have developed secondarily.

Lawrence, E. C. et al.

Clinical and Experimental Immunology 49:96-104, 1982.

Other support: American Lung Association and the National Institutes of Health.

From the Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Research Center of The Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

NEUTRAL GLYCOSPHINGOLIPIDS OF HUMAN ACUTE LEUKEMIAS

A methodology was introduced in this study which combines the sensitivity of high performance liquid chromatography with the specificity of exo- and endoglycosidases to study the neutral glycosphingolipids present in the malignant cells of 10 patients with acute leukemia. Results showed that acute leukemia cells contain very little or none of the more complex neutral glycosphingolipids that are found in normal leukocytes or chronic leukemia cells. Lymphoblasts, in particular, are rich in neutral glycosphingolipids with only one or two carbohydrate units. The most significant finding of this study was that, in contrast to normal leukocytes and chronic leukemia cells which have a single predominant tetraosylceramide species, acute leukemia cells (9 out of 10 patients analyzed) were found to have significant amounts of both globo (GalNAcβ1 Y 3Galα1 Y 4Galβ1 Y 4Glcβ1 Y lceramide) and neolactotetraosylceramide (Galβ1 Y 4GlcNAcβ1 Y 3Galβ1 Y 4Glcα1 Y lceramide). These results indicate that the composition of neutral glycosphingolipids in acute leukemia cells differs significantly from that found in normal or chronic leukemia cells.

Lee, W. M. F., Westrick, M. A. and *Macher, B. A.*

The Journal of Biological Chemistry 257(17):10090-10095, 1982.

Other support: National Institutes of Health, National Cancer Institute, Leukemia Research Foundation, and Cancer Research Funds of the University of California.

From the Cancer Research Institute, University of California, San Francisco.

GLYCOSPHINGOLIPIDS OF NORMAL AND LEUKEMIC HUMAN LEUKOCYTES

Studies on neutral glycosphingolipids and gangliosides of normal and leukemic human leukocytes were reviewed for this presentation. It can be seen here that two methodological approaches have been used to determine the structure and distribution of glycosphingolipids among human leukocytes: (1) those that utilize chemical and enzymatic tools to determine the complete structure, and (2) those that rely on indirect assays of detection. The former methods allow one to assign a unique structure to each compound, but they have two disadvantages: (1) they require relatively large quantities of materials and (2) minor components may be lost during the process of preparing homogenous compounds for analysis. Indirect methods which have been used are sensitive and rapid, and allow one to compare easily several samples, but they have the

disadvantages of being indirect, with structures assigned on the basis of comparison with standards. Examined for this review were: (a) the glycosphingolipid composition of various leukocyte populations, (b) the differences in glycosphingolipids found among subsets of these cells, (c) the possible use of these compounds as markers of differentiation, and (d) the changes in glycosphingolipid composition that occur with leukemogenesis.

Macher, B. A., Lee, W. M. F. and Westrick, M. A.

Molecular and Cellular Biochemistry 47:81-95, 1982.

Other support: National Institutes of Health, National Cancer Institute, Leukemia Research Foundation, and Cancer Research Funds of the University of California.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco.

NEURON-SPECIFIC ENOLASE AS AN IMMUNOCYTOCHEMICAL MARKER FOR THE DIFFUSE NEUROENDOCRINE SYSTEM IN HUMAN FETAL LUNG

Neuron-specific enolase (NSE) is an isoenzyme of the glycolytic enzyme enolase, which was originally considered to be restricted to neurons but has recently been shown to occur in some APUD cells. In this paper, the localization of NSE in the diffuse neuroendocrine system of human fetal lung is reported. Specifically, NSE-positive cells, singly or in groups, were demonstrated by antisera raised to human or rat NSE. Immunostained serial sections indicated that NSE-positive cells could also contain bombesin and /or 5HT-like immunoreactivity. At least three different cell types were identified containing (1) NSE, 5HT, and bombesin, (2) NSE and 5HT, and (3) NSE alone. After close consideration of the material presented here, it appears that NSE is a useful marker of the neuroendocrine system in the lung as well as in other tissues. Also, the lack of alternative simple and reliable techniques capable of identifying both cells and nerves means that the immunocytochemical localization of NSE is a valuable tool for the study of development, physiology, and pathology of this system.

Wharton, J., *Polak, J. M.*, Cole, G. A., Marangos, P. J., and Pearse, A. G. E.

The Journal of Histochemistry and Cytochemistry 29(12):1359-1364, 1981.

From the Department of Histochemistry, Royal Postgraduate Medical School, London, England, and the Clinical Psychobiology Branch, National Institutes of Health, Bethesda, MD.

IgE-DEPENDENT RELEASE OF LEUKOTRIENE C_4 FROM ALVEOLAR MACROPHAGES

Slow reacting substances (SRS) have been shown recently to be a family of peptidolipids called leukotrienes (LTC_4, LTD_4 and LTE_4) that are derived from arachidonic acid and are potent bronchoconstrictors *in vivo* and *in vitro*. In the study presented here, rat alveolar macrophages are shown to be the cells responsible for releasing the SRS. The ability of these macrophages to release SRS was tested initially by incubating cell suspensions for 20 min. with 1 μmol of calcium ionophore A23187,

in the presence of 5×10^{-4}M L-cysteine. In subsequent experiments, cell suspensions were stimulated with purified mouse monoclonal anti-DNP (dinitrophenyl) IgE antibody and DNP-human serum albumin. Results of these experiments show that (1) rat alveolar macrophages release SRS when stimulated non-specifically by the calcium ionophore A23187 in the presence of L-cysteine, and (2) IgE antibody and appropriate antigen cause alveolar macrophages to release SRS leukotriene, LTC_4. This demonstration that rat alveolar macrophages release SRS by an IgE-dependent mechanism raises the possibility that IgE-dependent release of mediators by alveolar macrophages may have a role in asthma or other immunologically mediated lung diseases.

Rankin, J. A. *et al. (Reynolds, H. Y.)*

Nature 297:329-331, 1982.

Other support: National Institutes of Health and Hoffman La-Roche Inc.

From the Department of Medicine and Pharmacology, Yale University School of Medicine, New Haven, and the John B. Pierce Foundation, New Haven, CT.

MONOCLONAL ANTIBODY ANALYSIS OF COMPLEX BIOLOGICAL SYSTEMS: COMBINATION OF CELL HYBRIDIZATION AND IMMUNOADSORBENTS IN A NOVEL CASCADE PROCEDURE AND ITS APPLICATION TO THE MACROPHAGE CELL SURFACE

In this sophisticated methodological paper, a procedure is described which greatly simplifies the collection of monoclonal antibody (MAb) libraries directed toward individual components of complex biological systems. For the study reported here, removal of previously recognized antigens with immunoadsorbent columns was combined with cell hybridization in a cascade which restricts the immunizing stimulus to previously unrecognized antigens. Specifically, in this report a cascade procedure was explored in connection with the identification of further macrophage-specific antigens. Peritoneal exudate cell membranes were detergent solubilized, and the previously identified common leukocyte antigen and heat-stable antigen which are shared with peritoneal exudate cells and lymphocytes were removed with MAb^2 immunoadsorbents before immunization for the hybridization experiment. Removal of the antigens was confirmed by radioimmunoassay and by the serological response to immunization. Serum antibodies to specific antigens were also measured to compare the efficacy of this procedure to immunization with either whole cells or MAb-coated cells. Two previously unknown macrophage-specific antigens of 32,000 and 110,000 M, were identified here. According to the authors, the precedure can be extended by arranging further immunoadsorbent depletions and cell fusions in a cascade series and is readily applicable to the monoclonal antibody analyses of many other multicomponent biological complexes.

Springer, T. A.

The Journal of Biological Chemistry 256(8):3833-3839, 1981.

Other support: U. S. Public Health Service.

From the Department of Pathology, Harvard Medical School, Boston.

MAC-1, 2, 3 AND 4: MURINE MACROPHAGE DIFFERENTIATION ANTIGENS IDENTIFIED BY MONOCLONAL ANTIBODIES

The Kohler-Milstein myeloma hybrid technique, which can enable the isolation of a monoclonal antibody recognizing a single antigenic determinant from an initial highly complex antigen, has given great impetus to the analysis of cell surface complexity. In the paper presented here, work done in the author's laboratory using this technique for the identification and study of macrophage antigens is reviewed. Four antigens — Mac-1,2,3 and 4 — have been identified by the corresponding monoclonal antibodies, Ml/70, M3/31, M3/38, M3/84 and M3/37. These antigens all appear to be on the macrophage cell surface on the basis of fluorescent and ^{125}I-labeling. ^{35}S-methionine incorporation into the polypeptides by the adherent fraction of thioglycollate-induced peritoneal exudate cells also suggests these antigens are synthesized by macrophages. The four different antigens defined in these studies are present on macrophages, but not lymphocytes, demonstrating the distinctiveness of macrophage cell surface architecture. Currently, the expression of these antigens on macrophages induced by other means and in different anatomical locations is being investigated. The monoclonal antibodies are also being used as probes to inhibit a panel of macrophage functions. In this way, it should be possible to link the molecular structures described here with specific macrophage cell surface activities.

Springer, T. A.

In: Förster, O. (ed.): *Heterogeneity of mononuclear phagocytes*, New York: Academic Press, 1980, pp. 37-46.

Other support: U. S. Public Health Service.

From the Department of Pathology, Harvard Medical School, Boston.

A SHARED ALLOANTIGENIC DETERMINANT ON Ia ANTIGENS ENCODED BY THE I-A AND I-E SUBREGIONS: EVIDENCE FOR I REGION GENE DUPLICATION

It has been known for a while that the I region of the major histocompatibility complex contains genes that control immune response and immune suppression to certain antigens and different determinants, and studies on these genes have led to the definition of a number of I subregions. In the study presented here, two rat monoclonal antibodies (MAb), M5/114 and M7/81, which have a very unusual type of crossreactive specificity for murine I region products, are characterized. These MAb detect polymorphic determinants present on B cells and activated T lymphocytes from mice carrying the H-2^b, H-2^d, H-2^k, H-2^r, and H-2^s haplotypes but not from mice carrying the H-2^a or H-2^f haplotypes. Antigenic site number determinations showed that the positive haplotypes can be divided into two groups. Mice bearing the H-2^b, H-2^d, and H-2^s haplotypes express a high number (40,000 to 80,000) of antigenic sites per B lymphocyte, and MAb plus complement can lyse B cells from these mice. In contrast, mice bearing the H-2^k and H-2^r haplotypes express a low number of antigenic sites. Spleen cells from mice carrying the latter haplotypes are not lysed with MAb and complement. Genetic mapping demonstrated that high and low expression map to the I-A and I-E subregions, respectively. The MAb detect an Ia specificity on I-A^b, I-A^d, I-E^d, and I-E^k molecules. These observations were confirmed using several different experimental approaches, i.e., cytotoxicity, fluorescent staining, competitive inhibition of MAb

binding, and 2-dimensional gel electrophoresis of immunoprecipitates. Results of this study provide immunologic evidence for homology between I-A and I-E antigens, and hence for gene duplication within the I region.

Bhattacharya, A., Dorf, M. E. and *Springer, T. A.*

The Journal of Immunology 127(6):2488-2495, 1981.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, and the Department of Pathology, Harvard Medical School, Boston.

NATURAL KILLER ACTIVITY IN THE PERITONEAL EXUDATES OF MICE INFECTED WITH *LISTERIA MONOCYTOGENES*: CHARACTERIZATION OF THE NATURAL KILLER CELLS BY USING A MONOCLONAL RAT ANTI-MURINE MACROPHAGE ANTIBODY (Ml/70)

Natural killer (NK) cells are mononuclear cells of disputed lineage that kill certain destructive cells in the body. In the paper presented here it can be seen that infection with *Listeria monocytogenes* (LM) leads to the generation of NK activity in peritoneal exudates. Specifically, maximum expression of NK activity first occurred on day two and remained high until day six after initial exposure to LM. When nylon wool nonadherent peritoneal exudate cells were examined by a single-cell cytotoxicity assay, the number of cells binding to YAC-1 target cells increased after infection as did their individual lytic capacity. A monoclonal rat anti-murine macrophage antibody (Ml/70), previously shown to recognize human NK cells, can be used also as a marker for murine NK cells. Utilizing Ml/70 and the fluorescence-activated cell sorter, selection of Ml/70-labeled mononuclear cells led to the enrichment of both NK and antibody-dependent cellular cytotoxicity. These Ml/70-positive cells had a distinctive morphology and contained granules on Wright-Giemsa staining. They were not phagocytic, did not contain nonspecific esterase, and lacked surface I-A^k, IgM determinants, complement receptors, and high levels of Thy 1.2.

Holmberg, L. A., *Springer, T. A.* and Ault, K. A.

The Journal of Immunology 127(5):1792-1799, 1981.

Other support: National Institutes of Health.

From the Department of Pathology, Harvard Medical School, Boston.

MACROPHAGE DIFFERENTIATION ANTIGENS: MARKERS OF MACROPHAGE SUBPOPULATIONS AND TISSUE LOCALIZATION

The characteristics of four distinct antigens which are present on macrophages, but not lymphocytes, are reviewed in this paper. In addition, two applications of anti-macrophage monoclonal reagents—their use in phenotyping macrophage subpopulations and in identification of macrophages in tissue sections—are described here. The major sections of this paper are devoted to MAC-1 ANTIGEN, IMMUNOADSORBENT-CELL HYBRIDIZATION CASCADES, MAC-2, 3 AND 4 ANTI-

GENS, and IDENTIFICATION OF MACROPHAGES IN TISSUE SECTIONS BY INDIRECT IMMUNOFLUORESCENCE. Overall, four macrophage antigens with distinct Mr and tissue distribution are identified in this work. Two of these, Mac-1 and Mac-3, are synthesized by all macrophage subpopulations examined thus far. However, Mac-2 seems to be preferentially associated with thioglycollate-elicited peritoneal macrophages. Ia antigens show a different pattern of expression. Therefore, macrophages can be defined into subsets with distinct antigenic phenotypes, as is the case for lymphocytes.

Springer, T. A. and Ho, M-K.

In: Mitchell, M. S. and Oettgen, H. F. (eds.): *Hybridomas in Cancer Diagnosis and Treatment*, New York: Raven Press, 1981, pp. 35-46.

Other support: U. S. Public Health Service.

From the Department of Pathology, Harvard Medical School, Boston.

RAT ANTI-MOUSE MACROPHAGE MONOCLONAL ANTIBODIES AND THEIR USE IN IMMUNOFLUORESCENT STUDIES OF MACROPHAGES IN TISSUE SECTIONS

The characteristics of five monoclonal antibodies (MAb) to macrophage antigens are summarized in this paper, which also contains a description of the use of one of these MAb for the localization of macrophages in frozen spleen sections. Of the five rat monoclonal antibodies to mouse macrophage surface antigens that were developed in the authors' laboratory by the myeloma-spleen cell hybrid technique of Kohler and Milstein, MI/70, which recognizes a phagocyte-specific antigen, Mac-1, is the most extensively studied. Two other antibodies, M3/31 and M3/38, precipitate a polypeptide termed Mac-2, which is also characterized here. In the related study, anti-Mac-1 was used to stain macrophages in spleen sections because Mac-1 seems to be expressed on macrophages irrespective of their state of differentiation and activation. To allow alignment of areas with Mac-1$^+$ cells with T-dependent areas of the spleen, adjacent sections were stained with M5/49, an anti-Thy-1 MAb. Results showed that T lymphocytes in the periarteriolar lymphatic sheath are intensely stained by anti-Thy-1 MAb. In contrast, few, if any, Mac-1$^+$ cells can be seen in these T-dependent areas. The simple method for the localization of macrophages described in this publication can be easily extended to other studies, especially with anti-Mac 1, which stains all macrophage subpopulations examined so far. In view of the vast body of information that can be gained from anatomical localization of lymphocyte subpopulations, these studies should provide much insight into the function, differentiation, and ontogeny of macrophages.

Ho, M-K. and *Springer, T. A.*

In: Hammerling, U., Hammerling, G. and Kearney, J. (eds.): *Monoclonal antibodies and T cell hybridomas*, New York: Elsevier/North Holland Biomedical Press, 1981, pp. 53-61.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

MAC-2, A NOVEL 32,000 M_r MOUSE MACROPHAGE SUBPOPULATION-SPECIFIC ANTIGEN DEFINED BY MONOCLONAL ANTIBODIES

The biochemical characterization and cell distribution of a 32,000 M_r antigen, termed Mac-2, are presented in this paper. Mac-2 is synthesized by and expressed on the surface of thioglycollate-elicited macrophages as shown by [^{35}S]-methionine and ^{125}I labeling. Unelicited peritoneal macrophages and macrophages elicited by protease peptone, Con A, LPS, and *Listeria monocytogenes* are either only weakly positive or negative. Therefore, Mac-2 expression is induced only by strong inflammatory stimuli and appears specific for mononuclear phagocyte subpopulations in a distinct stage of differentiation. Results of saturation binding experiments show that thioglycollate-elicited macrophages express 1.7×10^5 Mac-2 sites/cell. Thioglycollate-elicited macrophages are strongly absorptive for ^{125}I-labeled M3/38 MAb. Cell suspensions from spleen, bone marrow, thymus, and peripheral lymph node are > 99% Mac-2 negative by immunofluorescent flow cytometry. In contrast, thioglycollate-elicited macrophages are > 96% strongly positive for Mac-2. SDS-PAGE of [^{35}S]-methionine-labeled Mac-2 shows that thioglycollate-elicited macrophages synthesize 10- to 30-fold more Mac-2 than other peritoneal macrophage subpopulations, whereas all types of peritoneal macrophages synthesize and express on their surfaces similar amounts of the Mac-1 antigen. Mac-2 antigen is, therefore, induced in macrophages only in response to specific differentiative signals.

Ho, M-K. and *Springer, T. A.*

The Journal of Immunology 128(3):1221-1228, 1982.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Boston.

ONTOGENY OF MURINE MACROPHAGES: FUNCTIONS RELATED TO ANTIGEN PRESENTATION

Immature macrophage function contributes to the increased susceptibility of neonates to infection. In this paper, the immaturity of neonatal macrophage function is dissected into antigen presentation and three different effector components: cytotoxicity, antigen uptake and catabolism, and the production of the lymphostimulatory molecule interleukin-1 (also called thymocyte mitogenic protein or lymphocyte-activating factor). The uptake and catabolism of ^{125}I-labeled *Listeria monocytogenes* were equivalent in macrophages from adult and neonatal mice. However, interactions between macrophages from neonates, heat-killed *Listeria* organisms, and immune T lymphocytes were impaired, and no cytocidal macrophages capable of killing tumor cells were generated. Previous studies with cells from adult mice had established that the development of cytocidal macrophages required Ia-bearing, antigen-presenting macrophages and histocompatibility at I-A between macrophages and T cells. To circumvent this requirement for antigen-presenting macrophages, an assay was used in which lymphokine was added directly to the macrophages from neonates. Strong cytocidal activity resulted. Thus, these studies showed that macrophages from neonates present antigen poorly but can acquire cytocidal function provided that the need

for antigen-presenting function is bypassed. Similar conclusions were reached for the secretion of interleukin-1. In essence, all the data presented here indicate that the impairment of a number of macrophage functions in the neonates is due to a reduced number of Ia-positive macrophages.

Lu, C. Y. and *Unanue, E. R.*

Infection and Immunity 36(1):169-175, 1982.

Other support: National Institutes of Health and the March of Dimes.

From the Department of Pathology, Harvard Medical School, Boston.

CONTROL OF MACROPHAGE Ia EXPRESSION IN NEONATAL MICE—ROLE OF A SPLENIC SUPPRESSOR CELL

As reported in this paper, the control of macrophage expression of I region-associated antigens (Ia) in neonatal mice was studied by comparing responses of neonatal and adult mice to immune vs. nonimmune stimuli. Adults generated peritoneal exudates rich in Ia-bearing macrophages in response to i.p. injection of live *Listeria monocytogenes*, *Listeria*-immune T cells, and heat-killed *Listeria*, or a soluble mediator termed macrophage Ia-recruiting factor (MIRF). Neonates failed to respond to these stimuli. In contrast, both neonates and adults generated Ia-negative peritoneal exudates when stimulated with thioglycollate. There are three major new points that came out of these studies: (1) neonatal mice not only have a defect in their basal number of Ia-positive phagocytes but also fail to respond to the immune stimuli that generate exudates enriched for these cells; (2) there is a suppressor system operating in the neonate capable of significantly dampening the recruitment of Ia-positive macrophages—this suppressor system is also operant in some adult tissues such as bone marrow and the peritoneal cavity; and (3) the suppressor mechanism involves, at least, the phagocyte system by way of an indomethacin- and aspirin-sensitive step. Overall, it appears that this phagocytic line autoregulates its surface expression of Ia in both neonatal and adult mice. Since this mechanism becomes particularly pointed during early development, it could contribute to the lack of immunity during ontogeny.

Snyder, D. S., Lu, C. Y. and *Unanue, E. R.*

The Journal of Immunology 128(3):1458-1465, 1982.

Other support: National Institutes of Health and the March of Dimes.

From the Department of Pathology, Harvard Medical School, Boston.

SPONTANEOUS T-CELL LYMPHOKINE PRODUCTION AND ENHANCED MACROPHAGE Ia EXPRESSION AND TUMORICIDAL ACTIVITY IN MRL-lpr MICE

Selected macrophage functions in MRL/Mp-lpr/lpr (MRL-lpr) mice were evaluated for this report. Specifically, three macrophage functions were studied in MRL-lpr mice with autoimmune lymphoproliferative disease: surface expression of I-region-associated (Ia) antigens, tumor cytotoxicity, and interleukin-1 (IL-1) production. MRL-lpr mice had a significantly increased representation of Ia-positive macrophages

in the peritoneal cavity, compared to all normal strains of mice. In order to study the basis of this increase, thymocytes or splenocytes from MRL-lpr mice were transplanted intraperitoneally into normal mice. Three days later the recipient mice had peritoneal exudates rich in Ia-positive macrophages. The cells which induced this response were T cells which elaborated a lymphokine responsible for the recruitment of Ia-positive macrophages. In previous studies from this laboratory using mice, lymphokine was secreted only following the interaction of immune T cells with antigen. The resident macrophages of MRL-lpr mice were activated and killed tumor cells if triggered by an interaction with bacterial products, even without the addition of lymphokines. Secretion of IL-1 was normal. Results indicate that the diseased MRL-lpr mice are characterized by (i) activated T cells that spontaneously secrete macrophage stimulatory molecules, and (ii) activated macrophages that show both an increased expression of Ia and lymphokine-independent triggering of tumoricidal activity.

Lu, C. Y. and *Unanue, E. R.*

Clinical Immunology and Immunopathology 25:213-222, 1982.

Other support: National Institutes of Health and the March of Dimes.

From the Department of Pathology, Harvard Medical School, Boston.

VII. *Epidemiology*

MORTALITY IN MIDDLE-AGED SMOKERS AND NONSMOKERS

The relation of cigarette smoking to mortality was assessed in an 11-year follow-up study of 4,004 men and women, 35-54 years of age, who responded to urging to have multiphasic health checkups. Accounting for 48 other characteristics, both individually and in combination, failed to eliminate the association of smoking with mortality from all causes or with mortality from coronary heart disease. The smoker-to-nonsmoker mortality ratios, crude and adjusted respectively, were 2.6 and 2.1 for all causes and 4.7 and 3.6 for coronary heart disease. This analysis did not support the counterhypothesis that the association of cigarette smoking with mortality is secondary to some underlying characteristic.

Friedman, G. D., Dales, L. G. and Ury, H. K.

The New England Journal of Medicine 300(5):213-217, 1979.

Other support: Kaiser Foundation Research Institute.

From the Department of Medical Methods Research, Kaiser-Permanente Medical Care Program, Oakland, CA.

CHARACTERISTICS OF SMOKING-DISCORDANT MONOZYGOTIC TWINS

The Kaiser-Permanente Twin Registry of Oakland, CA, contains over 8,000 pairs of twins who volunteered their participation in a program of medical research on twins. In 1977 and 1978 a large health questionnaire was mailed to all twins aged 18 years and over. After the first mailing in June 1977, a reminder postcard was sent in October 1977 to nonresponding same-sex twins. In this questionnaire, each twin was asked about his or her own smoking habits and those of his or her cotwin. In the study presented here, the smoking habits of the 33 cigarette smokers in the smoking-discordant monozygotic (MZ) female pairs were compared with those of the 205 other MZ female cigarette smokers. Large and statistically significant differences were noted in some measures of smoking intensity. Cigarette smokers who had an MZ nonsmoking cotwin tended to start smoking later and to smoke fewer cigarettes. This may explain, in part, the smaller difference in CHD occurrence between smokers and nonsmokers within smoking-discordant twins than between smokers and nonsmokers in the general population. Additional comparisons were also made between the discordant smokers and their nonsmoking cotwins. Results of these comparisons show that, with regard to coronary heart disease (CHD) risk factors, the discordant smokers were leaner and consumed more alcohol than their nonsmoking cotwins. While these traits are associated with a lower risk of CHD, smokers also tended to be less educated and reported less exercise and concern about physical fitness, consistent with higher risk. In conclusion, the data on a limited number of smoking-discordant female MZ twins suggest that, even with genetic identity, twins who differ in one characteristic may differ in other characteristics relevant to the outcome under consideration.

Friedman, G. D. et al.

In: Gedda, L., Parisi, P., and Nance, W. E. (eds.): *Twin Research 3: Epidemiological and Clinical Studies,* New York: Alan R. Liss, Inc., 1981, pp. 17-22.

Other support: National Heart, Lung and Blood Institute.

From the Department of Medical Methods Research and Department of Medicine, Kaiser-Permanente Medical Care Program, Oakland; University of California, Berkeley; and the Department of Epidemiology and International Health, University of California, San Francisco.

STRUCTURAL ANALYSIS OF SMOKING, ALCOHOL USE, AND PERSONALITY FACTORS IN MZ AND DZ TWIN PAIR RELATIONSHIPS

This study presents an extension of the testing of twin type comparability to a multivariate situation. Using data drawn from the Finnish Twin Cohort as the study population, smoking characteristics, alcohol use patterns, and personality factors of monozygotic (MZ) and dizygotic (DZ) twin pairs concordant and discordant for frequent heavy alcohol use were described. The effect of constraining covariance relationships in individuals of either twin type was tested to estimate comparability of pairwise structures. Also, the covariance relationships in individual twins were compared to those of singletons. For such analyses, the general model for the estimation of linear structural equation systems by maximum likelihood methods was applied to the Finnish Twin Cohort study data. Results showed that the differences between heavy-

and light-drinking individuals were most clear-cut in singletons. They differed in the mean smoking levels, and the extraversion and neuroticism scores. The same result was obtained in DZ pairs discordant for heavy alcohol use. In discordant MZ pairs, however, the smoking differences were observed but the personality factor differences were seen in the neuroticism and life dissatisfaction scales, suggesting that the basis of discordance in MZ twins may differ from that in DZ pairs and singletons. The proportion of discordant pairs was 29% in MZ and 37% in DZ pairs, less than the expected value. Multivariate analysis of differences in MZ and DZ discordant pairs confirmed the results from the univariate analyses, the relative significance of neuroticism in MZ pairs becoming weaker and statistically nonsignificant. Smoking, neuroticism, and life dissatisfaction, independent of genetic factors, seem to be indicators of processes leading to heavy alcohol use.

Langinvainio, H., Kaprio, J., Koskenvuo, M., and Tarkkonen, L. *(Rantasalo, I.)*

In: Gedda, L., Parisi, P. and Nance, W. E. (eds.): *Twin research 3: epidemiological and clinical studies*, New York: Alan R. Liss, Inc., 1981, pp. 23-35.

From the Department of Public Health Science, University of Helsinki, Helsinki.

CIGARETTE SMOKING, USE OF ALCOHOL, AND LEISURE-TIME PHYSICAL ACTIVITY AMONG SAME-SEXED ADULT MALE TWINS

The relationships of cigarette smoking, alcohol use and leisure-time physical activity among adult male twin participants in a Finnish population study are presented here. For this epidemiological study, questionnaire responses from 1,537 monozygotic (MZ) and 3,507 dizygotic (DZ) male pairs aged 18 and over were analyzed in terms of combinations of the three factors and the relationship of these factors to each other in relation to the twin pair situation. To do this, three factor analyses and an overall cluster analysis were carried out. Results of these tests showed that the physical activity factor means were almost constant with age, but there was a decrease with age in alcohol consumption; for the smoking factor, there was a steady increase with age until 50-54 years, after which a slight decrease occurred. The correlation coefficients between the factors in the whole series showed a high correlation between cigarette smoking and use of alcohol, and small negative correlations for physical activity and cigarette smoking and for physical activity and use of alcohol. In the cluster analysis, eight clusters were found to be stable in group-regroup situations with over 90% of members remaining in the same cluster from one analysis to another. As to twinship, both MZ and DZ twin pair members were in the same cluster much more often than expected, but the MZ-DZ overall difference was relatively small. The highest MZ/DZ ratios of observed to expected clustering rates were in two clusters: A) cluster no. 7, which had persons with a high mean degree of leisure-time physical activity; and B) the very small cluster no. 8, which had a very high mean alcohol use.

Kaprio, J., Koskenvuo, M. and Sarna, S. *(Rantasalo, I.)*

In: Gedda, L., Parisi, P. and Nance, W. E. (eds.): *Twin research 3: epidemiological and clinical studies*, New York: Alan R. Liss, Inc., 1981, pp. 37-48.

From the Department of Public Health Science, University of Helsinki, Helsinki.

CORONARY-PRONE BEHAVIOR IN ADULT SAME-SEXED MALE TWINS: AN EPIDEMIOLOGICAL STUDY

In this attempt to identify familial and environmental components of coronary-prone behavior patterns, the responses from 5,419 male twin pairs in the Finnish Twin Cohort to a 1975 questionnaire were investigated in several different ways. To begin with, the postal questionnaire study provided data on zygosity, smoking, alcohol use, leisure-time physical activity, weight, height, and drug usage. Psychosocial factors such as marital status, occupation and occupational history, changes of residence and employment, extroversion and lability, and type A behavior were also studied, as well as various symptoms and history of disease. Type A behavior pattern was measured by the rating scale developed by Bortner. Results of this study showed that the intraclass correlations were 0.251 for monozygotic (MZ) pairs and 0.052 for dizygotic (DZ) pairs. The heritability estimates were higher in younger than in older age groups, and the proportion of A-type concordant pairs also showed an age trend. While the proportion of MZ pairs among A-type concordant pairs was greater than among B-type concordant pairs, the difference was not statistically significant. In this study, an association in men between A-type behavior pattern and angina pectoris on the Rose questionnaire was found. Moreover, the discordant pair analysis presented here showed that there were some environmental factors clearly associated with coronary-prone behavior. As of now, A-type behavior has been shown to be an independent risk factor for different manifestations of coronary heart disease (CHD). Since some psychosocial factors, such as marital status and social class, which were found to correlate with A-type behavior in this study, are known to be associated with CHD in Finland, it seems reasonable that the relationship of A-type behavior, psychosocial factors, and CHD should be investigated further.

Koskenvuo, M., Kaprio, J., Langinvainio, H., Romo, M. and Sarna, S. *(Rantasalo, I.)*

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From the Department of Public Health Science, University of Helsinki, Helsinki.

FINNISH TWINS REARED APART: PRELIMINARY CHARACTERIZATION OF REARING ENVIRONMENT

This paper presents some characteristics of the rearing environment of 478 Finnish-speaking, adult, like-sexed twin pairs raised apart from the age of 10 or less. The Finnish Twin Cohort Study provided the raw data base for this study, and twinship was confirmed by a questionnaire study in 1975 that covered health-related items and standardized measures of morbidity. In addition to these questions, a number of other aspects were considered: whether the twin pair lived together and, if not, at what age separation had occurred. The present frequency of intrapair contact, birth order, and handedness were also investigated and questions directed to zygosity assessment were included. Later, during November 1979—January 1980, a new questionnaire on their childhood environment went out to the 478 twin pairs in the test group and three corresponding control groups, which were formed to assess which aspects of the rearing environment, personality factors, and childhood medical history of the study sample differed from those of twins of the same age and sex. Listings for this study are

given by age of separation (groups I-IV) and by birth year and sex. Results of this study showed that the intrapair correlation of rearing environment varied greatly as it appeared from variables such as age at separation, family members, school, friends, living place, intrapair contact frequency, and education and occupation of rearing parents. Moreover, the cause of separation, based on self-report, seemed to be fairly often associated with some psychosocial pathology. The separation of members of a twin pair may also mean intrapair selection. Further assessment and comparisons with singletons from the general population and with psychiatric outpatients are ongoing for this study.

Langinvainio, H. *et al. (Rantasalo, I.)*

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From the Departments of Public Health Science and Psychiatry, University of Helsinki, Helsinki.

CANCER IN ADULT SAME-SEXED TWINS: A HISTORICAL COHORT STUDY

In this attempt to investigate the feasibility of utilizing the twin method as a case-control type of study, a historical record-linkage cohort study between the Finnish Twin Cohort Study and the Finnish Cancer Registry was carried out. The Finnish Twin Cohort was created in 1974 from the computerized Central Population Registry, while the Finnish Cancer Registry is a population-based, national registry in operation since 1953. It is considered to be rather complete with respect to incident cases of cancer in Finland. For this study, persons included in both registries were identified by comparing by computer the personal identification numbers (a 10-digit unique code assigned to each resident in Finland) of the two registries. The comparison covered the years 1967-1976. The twin record linkage yielded the observed numbers of cancers of different types. Age and sex-specific person-years at risk were calculated separately for the twin population and the singleton group. Also, person-years at risk were calculated for cotwins of cancer probands. Results of this study showed that the total cancer morbidity in the twin population was lower than expected for both men and women. The relative risk for all cancers was 0.77 for men and 0.72 for women. In the singleton population, the relative risk for men was slightly over unity. In this study, the ratio of the observed to expected cancer morbidity closely reflected the standardized (total) mortality ratios for the same calendar years, suggesting that the lower-than-expected cancer morbidity may have a background in common with the lower-than-expected mortality. Also, the low concordance rate found in this study suggests that it may be fruitful to study the environmental exposure of cancer-discordant MZ and DZ twins.

Kaprio, J. *et al. (Rantasalo, I.)*

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From the Department of Public Health Science, University of Helsinki, and Finnish Cancer Registry, Helsinki.

SLEEP DISORDERS IN RELATION TO CORONARY HEART DISEASE

Evidence from an American study indicating a relationship between sleep time and mortality, including death from coronary heart disease (CHD), led to a Finnish Twin Cohort study on the relationship between sleep time and CHD. In the epidemiological paper presented here, the sleeping time distributions in the U.S.A. taken from the Kripke study were compared to data obtained in 1975 from 5,419 Finnish adult men, and age-standardized proportions were computed for men aged 30 and over. Results showed, first of all, that the proportion of men sleeping 9-10 hours or more is higher in Finland than in the United States. They also showed that short (less than six hours) or long (more than 10 hours) sleepers had significantly more complaints relating to CHD than those who slept for seven-eight hours per night. Shortened sleep was especially related to angina pectoris and pain of possible infarction, and correlated with aging, poor sleep quality and/or Type A behavior pattern score. Long sleep, on the other hand, was correlated with good subjective sleep quality, but this group had the highest incidence of diagnosed myocardial infarction. This relationship held after statistically controlling for many possible confounders such as hypertension, drug and alcohol use, smoking and Type A behavior pattern. In addition, the cardiovascular physiology and pathophysiology of sleep is reviewed here and the relationship of some specific sleep disorders to CHD is discussed.

Partinen, M. *et al. (Rantasalo, I.)*

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From the Departments of Neurology, Physiology and Public Health Science, University of Helsinki, Helsinki.

MULTIVARIATE LOGIT ANALYSIS OF CONCORDANCE RATIOS FOR QUALITATIVE TRAITS IN TWIN STUDIES

This statistical paper presents a new approach for the analysis of a certain type of twin data. The model that is applied in this paper, the logit model, is analogous to the widely used log-linear model for contingency table analysis. This model, which permits testing of interaction effects before estimating the main effects of the study variables, may also be easily extended to four-way or even more complex tables, though the testing procedures and sequential hypothesis testing becomes increasingly demanding. In the example used for this study, data from a cross-national study of cigarette smoking among adult twin pairs in two countries was used. The multivariate assessment of genetic factors in relation to other factors was carried out by logit analysis of concordance ratios by analyzing three variables, zygosity, sex, and country, at the same time. Thus, the effect of sex and country on zygosity in the smoking trait could be controlled. For cigarette smoking, the present results indicate that in both countries the zygosity effect is significant, and independent of country and sex. Although not presented here, the results held for both current and ever cigarette smoking as well as for smoking over one pack a day either currently or ever. For the heavy smokers, the zygosity effect had a significant interaction with sex. A significant zygosity effect implies greater concordance for the trait among MZ than DZ pairs and is due to the identical genome and probably greater common environment of MZ twin pairs.

Kaprio, J., Sarna, S., and Koskenvuo, M. *(Rantasalo, I.)*

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From the Department of Public Health Science, University of Helsinki, Helsinki.